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Chromosomal Localization of the Human Elastin Gene

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SUMMARY

mRNA isolated from fetal human aorta was used to synthesize cDNA that was cloned into the *Pst*I site of pBR322. The recombinant clones were screened with an authentic sheep elastin cDNA, and one human clone that hybridized strongly was isolated and characterized. The 421-*base pair* (bp) insert of this human clone was sequenced by the dideoxy method, and the DNA sequence showed strong homology to the nontranslated portion of the sheep elastin cDNA. This result unequivocally identified the human clone, designated pcHEL1, as an elastin clone. Plasmid pcHEL1 labeled with [^{3}H] nucleotides was used in in situ hybridization experiments utilizing normal metaphase chromosomes and also with cells carrying a balanced translocation between chromosomes 1 and 2: 46,XY,t(1;2)(p36;q31). The results strongly suggest that the elastin gene is localized to the q31→qter region of chromosome 2.

INTRODUCTION

The elastic properties of many tissues of the vertebrate body such as the lung and larger arteries are due largely to the presence in the extracellular matrix of

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elastic fibers that are composed primarily of the protein elastin. Within the mature elastin fibers, the individual polypeptide chains are covalently connected by crosslinkages derived from the oxidation of lysine residues [1–5]. A polypeptide, designated tropoelastin, with a mol. wt. of about 72,000 has been isolated from the aortas of copper-deficient and lathyritic animals [6–8] and appears to be a soluble intermediate in the biosynthesis of the insoluble elastin fiber [9–17]. A significant portion of the amino acid sequence of porcine tropoelastin has been determined, although the isolated, sequenced tryptic peptides have not been ordered [18]. Cell-free translation of elastin mRNA has demonstrated that tropoelastin is the primary translation product [19–23].

A chick elastin cDNA clone containing 3′ nontranslated sequences [24] has been used to identify and to estimate the relative amounts of elastin mRNA in the developing chick aorta by blot hybridization. A single mRNA species of 3.5 kilobases (kb) hybridized to the cDNA probe, and this species increased greatly between day 7 and day 14 of development. When these levels were compared to functional elastin mRNA measured by translation in a rabbit reticulocyte lysate system and to the rate of elastin synthesis in freshly isolated aortas of various ages incubated in vitro, the results suggested that the changes in elastin synthesis seen during development are governed by the elastin mRNA content of the aorta [25–26]. Similar results were found when the levels of functional elastin mRNA in the developing sheep lung and ligamentum nuchae were compared to the rates of elastin synthesis in the tissues [16–17].

Tropoelastin contains about 830 amino acid residues, and, therefore, approximately 1,000 nucleotides of the 3.5-kb elastin mRNA should be untranslated. This was confirmed by DNA sequence analysis of a 1,300-bp sheep elastin cDNA clone [27], which showed that these untranslated sequences were located at the 3′ end. This clone, pcSEL1, also contained translated sequences, including some which encoded amino acid sequences partially homologous to those previously identified in porcine tropoelastin [18].

We report here the construction and characterization of a 421-bp human elastin cDNA clone containing sequences from the 3′ untranslated region that show strong homology to the sheep elastin cDNA. This human clone, pcHEL1, was used in in situ hybridization experiments to identify the human chromosome containing the elastin gene. These experiments localized the elastin gene to the q31→qter region of chromosome 2.

MATERIALS AND METHODS

Construction of cDNA Clones

mRNA was isolated from 26-week fetal human aortas using 8 M guanidinium HCl as described [17], since this tissue has been shown to be particularly rich in elastin mRNA in other species [24, 26]. Very briefly, poly (A+) mRNA recovered by chromatography on oligo (dT) cellulose [19] was used to synthesize blunt-ended cDNA molecules by the successive application of AMV reverse transcriptase, DNA polymerase, and Sl nuclease [28]. The cDNA was fractionated on a 15%–30% sucrose gradient, and molecules greater than 700 bp were tailed with dCTP using terminal transferase and hybridized to *Pst*I-cut PBR322 tailed with dGTP as described [24]. The recombinant plasmids were

used to transform *E. coli* MC 1061, and transformants were selected by growth on agar plates containing 5 μg/ml tetracycline.

Screening of Transformed Colonies

The tetracycline-resistant colonies were transferred to agar plates containing 20 μg/ml of ampicillin and incubated overnight at 37°C. The ampicillin-sensitive, tetracycline-resistant colonies were screened by the colony hybridization method of Grunstein and Hogness [29] using pcSEL1, which was nick-translated with ^{32}p to a specific activity of 4 × 10^8 cpm/μg DNA using the kit supplied by Bethesda Research, Gaithersburg, Md. Hybridization was carried out overnight at 42°C in 70% formamide, 0.4 M NaCl.

DNA Sequencing

Transformed bacteria were grown in 1 liter of M-9 medium, and plasmids were amplified by the addition of chloramphenicol to a final concentration of 170 μg/ml. Plasmid DNA was isolated by the alkaline method of Birnboim and Doly [30], followed by equilibrium centrifugation in CsCl, then restricted with *Pst*I, and the fragments isolated by electroelution after electrophoresis in 1% agarose gels. The DNA was extracted with phenol/chloroform and then cloned into the *Pst*I site of the phage M13mp8 [31]. The restriction fragment derived from the cDNA insert now contained in the recombinant phage was sequenced by the Sanger dideoxy method [32] using a universal primer of 17 nucleotides (Collaborative Research, Cambridge, Mass.).

Chromosome Preparation and in Situ Hybridization

Metaphase chromosome spreads were prepared from peripheral blood lymphocytes of normal, 46,XY males using standard techniques. Chromosome preparations were also made from a fibroblast line, GM1230, obtained from the human genetic Mutant Cell Repository (Camden, N.J.). These cells carry a balanced translocation between chromosomes 1 and 2: 46,XY,(1;2)(p36;q31). Air-dried slides were permitted to age in the cold (4°C) for at least 1 week prior to their use in the mapping studies.

In situ hybridization studies were performed using a protocol modified from several in the literature [33, 34]. Air-dried metaphase chromosome preparations on glass slides were used 1–3 weeks after preparation. Slides were treated with RNAse to remove any chromosomally bound RNA. The slides were washed free of RNAse and then dehydrated through an alcohol series. Chromosomal DNA was denatured by immersing the slides in 2 × SSC/70% formamide at a temperature of 70°C, followed by rapid transfer through an alcohol series of dehydration.

Probe DNA was ^{3}H-labeled by nick-translation to a specific activity of 4 × 10^7 cpm/μg according to the protocol described by Lai et al. [35], and the DNA was separated from labeled nucleotides by chromatography on a Sephadex column. Carrier salmon sperm DNA was added to an excess of 1,000×, and the DNA was ethanol precipitated. The DNA was resuspended in the hybridization mix, which consists of 25% formamide/2 × SSC/10% dextran sulfate, pH 7.0. Probe DNA was denatured for 5 min at 70°C, quickly chilled in ice, and added to the slides at a concentration of 0.05 μg/ml. Coverslips were placed on the slides, which were put in moist chambers and hybridized for 18 hrs at 37°C. After hybridization, the slides were extensively washed at 39°C to remove nonspecifically bound labeled DNA and were dehydrated through an alcohol series. Slides were dipped in liquid nuclear track emulsion (Kodak NTB-2), stored in dark boxes, and developed at appropriate intervals. Slides were banded using a modified Wright's Giemsa protocol [36] and analyzed under the microscope.

Metaphase spreads were selected with good chromosome morphology and limited background grains. The location of specific grains was noted using an idiogram from Yunis et al. [37].

RESULTS

DNA Sequence Analysis of Human Elastin cDNA Clone, pcHEL1

cDNA clones were constructed using mRNA isolated from human fetal aortas as described in detail in MATERIALS AND METHODS. Approximately 1,000 penicillin-sensitive, tetracycline-resistant transformants were screened by in situ hybridization [29] using the sheep elastin cDNA clone, pcSEL1 [27], as probe. Only one clone hybridized strongly to the probe, even though it had been expected that this tissue was synthesizing appreciable quantities of elastin. The positive recombinant plasmid was restricted with *Pst*I and the fragments analyzed by electrophoresis in 1% agarose gels that showed that it contained an insert approximately 420 bp in size (gel not shown). To prove the identity of the insert contained in the recombinant plasmid, the insert was recovered by electroelution from agarose gels, cloned into the *Pst*I site of the M13 phage, mp8 [31], and sequenced by the Sanger dideoxy method [32]. Because of the moderate size of the insert, both strands could be sequenced in their entirety. The sequence of pcHEL1 is shown in figure 1, which also displays the homologous region of pcSEL1. As readily seen by inspection, there is a marked degree of homology between the human and sheep sequences in this 3′ untranslated region. The reason for this strong conservation of nucleotide sequence is not readily apparent but is suggestive of a regulatory function. This strong sequence homology permits us to identify unequivocally pcHEL1 as a human elastin clone. DNA sequence analysis of the homologous region of the sheep gene has demonstrated that this region of the sheep cDNA is located in a continuous exon unbroken by intervening sequences. If, as seems likely, this is the case in the human gene, the 421-bp human cDNA should be an excellent hybridization probe.

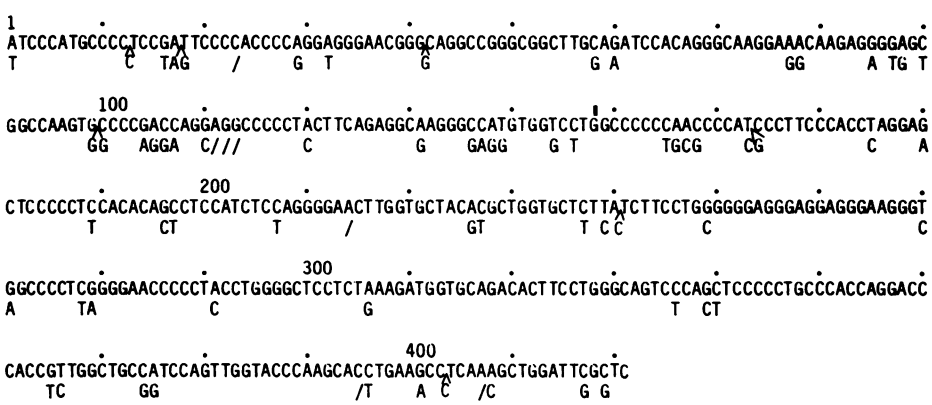

FIG. 1.—DNA sequences of the human elastin cDNA clone, pcHEL1, and the corresponding region of the sheep elastin cDNA clone, pcSEL1 [27]. The human clone is printed in its entirety, and only the differences in the sheep clone are printed below it. (/) = deletion of base in sheep clone; (^) = insertion in sheep clone.

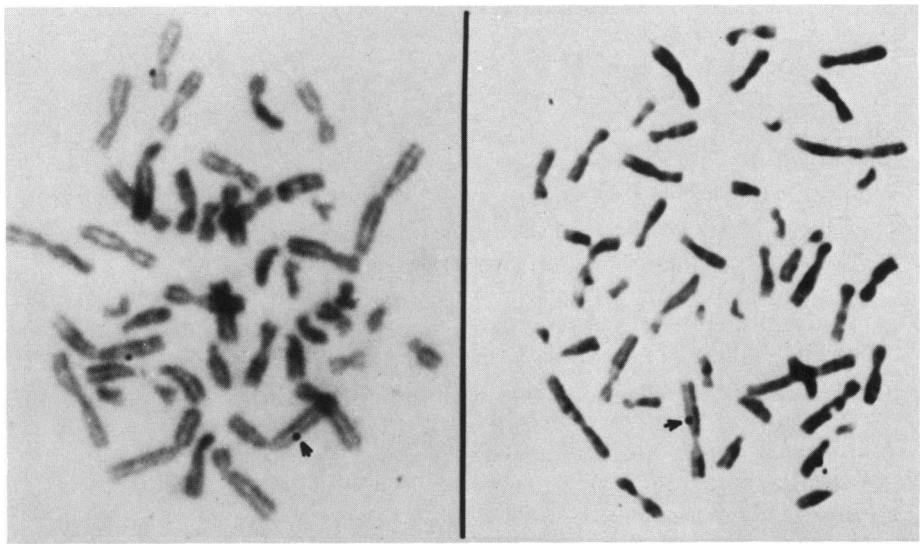

FIG. 2.—Representative autoradiographs from in situ hybridization of ^{3}H-labeled pcHEL1 elastin probe to metaphase chromosomes from a normal male (46,XY). *Arrows* indicate grains located on the 2q21→q33 region in two metaphase spreads.

Chromosomal Localization of Elastin Gene by in Situ Hybridization

Three independent in situ hybridization experiments using normal human metaphase chromosomes were carried out. Representative autoradiographs are illustrated in figure 2, and the results of the individual experiments are tabulated in table 1. The predominant site of hybridization in each experiment was the long arm of chromosome 2 (2q) on which 17%, 13%, and 27% of the total grains were located in the three experiments. The number of grains localized to 2q represented at least twice the number on any other chromosomal seqment of similar length in any given experiment, and no other region of the genome showed any consistent preferential hybridization with the elastin probe, suggesting that a single elastin locus is located on the long arm of chromosome 2. A total of 199 normal metaphase chromosome spreads were

TABLE 1

HYBRIDIZATION OF THE ELASTIN PROBE TO NORMAL HUMAN METAPHASE CHROMOSOMES

Experiment	No. metaphases	No. grains	2q chromosomal grains (q11→qter)
1	100	206	35/206 = 17.0%
2	56	82	11/82 = 13.4%
3	43	75	20/75 = 26.7%
Total	199	363	66/363 = 18.2%

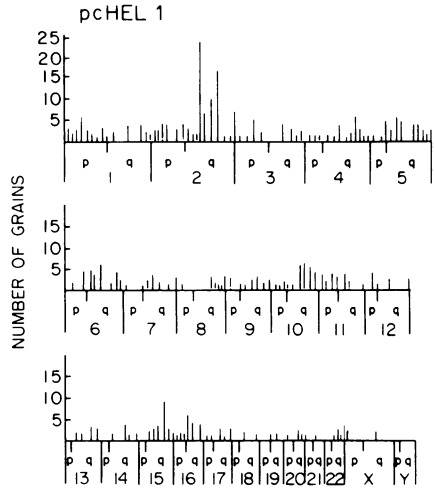

FIG. 3.—The histogram shows the distribution of silver grains on labeled chromosomes from 199 normal (46,XY) metaphases hybridized with the tritium-labeled pcHEL1 elastin probe. *The abcissa* represents the chromosomes in their relative size proportion; *the ordinate* shows the no. silver grains. Sixty-one percent of the grains on chromosome 2 were in the segment 2q21→2q33, suggesting localization of the human elastin gene to this region.

analyzed in which 363 grains were on chromosomes. Of the 363 grains, 66 (18.2%) were located on 2q from qll→qter. The finding that 18.2% of all grains are localized to 2q, a region that represents only 5% of the haploid autosome length, is highly significant ($P < .001$). The distribution of all grains on chromosomes from the combined three experiments is shown in figure 3 with 2q21→2q33 representing the segment with the majority of 2q hybridizations (61% of all grains on 2). These results suggest regional mapping of the elastin gene to the segment 2q2→2q3.

Additional in situ hybridization studies using the 1;2 translocation, 46XY,t(1;2)(p36;q31), were undertaken to map the elastin gene to a smaller segment of 2q. A total of 123 metaphases were examined in which 204 grains were on chromosomes (table 2). Of these 204 grains, 27 (13.2%) were on the long arm of the normal chromosome 2 and 20 (9.8%) were on the involved chromosome 1 in the 2q segment translocated to its short arm. A representative autoradiograph showing a partial metaphase involving the translated chromosome ($1p^+$) is illustrated in figure 4. Weak hybridization, 3 grains (1.5%) of the 204 grains, were found on the long arm of the $2q^-$ chromosome. Thus, the $2q^-$

TABLE 2

HYBRIDIZATION OF THE ELASTIN PROBE TO GM1230 CHROMOSOMES t(1;2)

No. metaphases	No. grains	Grains on normal 2q	Grains on 1p+
123	204	27/204 = 13.2%	20/204 = 9.8%

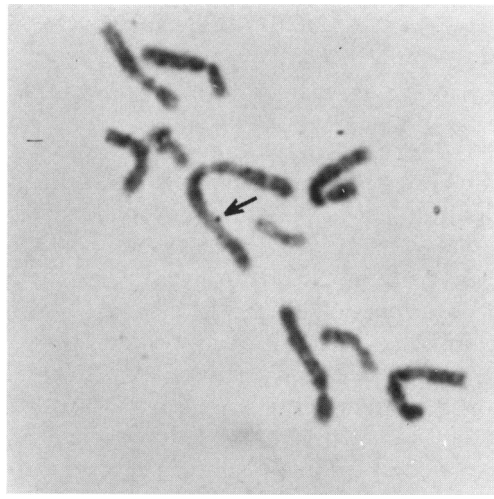

FIG. 4.—Representative autoradiograph from in situ hybridization of ^{3}H-labeled elastin probe, pcHEL1, to the cell GM1230. Partial metaphase demonstrating silver grain on 1p+ chromosome in the region corresponding to 2q31 (*arrow*).

chromosome no longer contains a specific site for the elastin gene and these grains represent nonspecific hybridization. These results suggest that the elastin locus was translocated to the 1p+ chromosome and, hence, maps in the q31→qter region of chromosome 2.

DISCUSSION

A 421-bp cDNA clone containing sequences complementary to a portion of the 3′ nontranslated region of human tropoelastin mRNA has been constructed and characterized. Although this clone contains no translated regions of mRNA, comparison of the DNA sequence to the nontranslated region of a previously characterized sheep cDNA clone that contains translated sequences unambiguously confirmed the identity of this human cDNA as the first cloned sequence corresponding to human tropoelastin mRNA. Furthermore, because of the size of the clone and because of the likelihood that the sequences are contained in a single exon, it proved to be an excellent probe for in situ hybridization.

Technical improvements in chromosomal in situ hybridization have permitted the regional assignment of numerous unique gene loci and anonymous DNA segments [33, 34]. We have used this technique to determine the chromosomal location of the human elastin gene utilizing a human cDNA clone as probe and metaphase chromosome preparations from normal individuals. Further sublocalization of the gene was achieved with the use of chromosome preparations from a fibroblast cell line carrying a balanced translocation involving 1p and 2q. Such chromosomal rearrangements have been used extensively in somatic cell genetics for deletion mapping [38], gene-dosage studies [39], and construction of somatic cell hybrids in which segregation of the relevant in-

volved chromosome can be correlated with the presence or absence of a particular gene [40]. Our present study suggests that these chromosome rearrangements are also valuable tools for gene mapping using chromosomal in situ hybridization.

In addition, our results suggest that caution should be exercised in the use of the in situ technique for precise chromosomal-band assignments in gene-mapping studies. The rather wide distribution of the silver grains located on chromosome 2 (fig. 3) is presumed to be due to an inherent technical limitation that precludes precise resolution and localization of the grains. This is not surprising as all localizations are done visually and chromosome 2 banding landmarks are often difficult to assign with precision because of the considerable amount of variation in the compaction of the 2q11→q31 region. Grains visually localized to 2q21 might actually be further down the long arm. Thus, the technique is useful for determining the chromosomal region containing the gene, but not the exact chromosomal band or subband. The precision of gene localization by in situ hybridization may be improved, however, by the judicious use of informative structural rearrangements of the involved region. Thus, by virtue of the hybridization of the segment translocated to the $1p^+$ chromosome, we were able to localize the elastin gene to a smaller region, q31→qter, of the long arm of chromosome 2. More precise band assignment of the elastin gene must await analysis using other, as yet to be described, informative rearrangements of 2q: those with breakpoints in the 2q31→qter region. Chromosome 2 is a comparatively unmapped region of the human genome with relatively few genes having been assigned to it [41]. The localization of the elastin gene together with future studies on restriction fragment length polymorphisms within or adjacent to the elastin gene should provide an important genetic marker for future linkage studies involving chromosome 2.

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COUNSELING STRATEGIES FOR PHYSICIANS program. Place: Oahu, Hawaii. Date: March 24–29, 1986. Registration: $325. Program approval: 17 hours AMA/PRA Category 1; AAFP Prescribed. Further information: Texas: 1-800-392-4900-737, Program Code 975 DLB; outside Texas: 1-800-332-8747. Summary: "Counseling Strategies for Physicians" is designed to introduce the latest techniques and research findings on matters that influence doctor-patient relationships. Faculty will discuss social change, family violence, addiction, and human sexuality as considerations in dealing with patients. They will present problems likely to be encountered with specific groups of clients such as teen-agers, minority groups, aging clients, and pregnant women. The program is being sponsored by The University of Texas at Tyler, Department of Social Sciences, in cooperation with The University of Texas Health Science Center at Houston, Division of Continuing Education.